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Carcinogenicity and Pesticides: Principles, Issues, and Relationships. Biological Issues in Extrapolation

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16. ABSTRACT

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Principles, Issues, and Relationships

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Chapter 9

Biological Issues in Extrapolation

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Approximately 41% (26/63) of the pesticides evaluated in the chronic toxicity and carcinogenicity studies of the National Toxicology Program (NTP) showed varying degrees of carcinogenicity. Since those chemicals nominated to the NTP for carcinogenicity studies usually represent a sampling of potentially "problem" chemicals, this ratio (i.e., 41%) does not implicate the actual percentage of carcinogenic chemicals among all pesticides. In general, results from epidemiological studies are of limited value in prevention and have been done on few pesticides. Furthermore, it is impossible to conduct chronic toxicity/carcinogenicity studies in humans. Therefore, laboratory animals must be utilized as surrogates, at least for the foreseeable future. To assess potential risks of chemicals to humans, extrapolation of the findings in laboratory animals to possible health effects in humans is inevitable. Several important biological issues must be considered in such a process; these include extrapolation between doses, species (including strain and sex), routes of administration and exposure regimens (e.g., intermittent vs constant rate). While there is no perfect surrogate for humans in the evaluation of carcinogenic potential and other toxicities of chemicals and other agents, rodents still represent the best models. Any imperfection in such a process (i.e., hazard identification and risk assessment of any given chemical or other agent) must be taken into consideration during extrapolation. The state-of-the-art technique or tools, such as physiologically based pharmacokinetics/computer modeling, should be considered and utilized judiciously to minimize the

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> intrinsic imperfections in the process. The continuing refinement and improvement of the entire process (from selection of chemicals for testing, experimental design and conduct, interpretation and analysis of data, interpolation and extrapolation, to risk assessment) is essential as scientific advances are made in these areas. Other important issues related to hazard identification and risk assessment include multiple chemical exposure as well as multiple route exposure; these must be incorporated into the process as information and tools become available.

In a recent review article (1), the Council on Scientific Affairs of the American Medical Association reported their findings on the cancer risk of pesticides in agricultural workers. Their conclusions were: (a) The primary hazard of pesticide exposure is the development of acute toxic reactions as a result of dermal contact with or inhalation of a relatively large dose. The effects usually are manifested within minutes or hours of contact; (b) Epidemiological studies offer only limited evidence at best that pesticides may be carcinogenic; (c) A large number of pesticidal compounds have shown evidence of genotoxicity or carcinogenicity in animal and in vitro screening tests, but no pesticides -- except arsenic and vinyl chloride (once used as an aerosol propellant) -- have been proved definitely to be carcinogenic in man; (d) With few exceptions, the long term (e.g., carcinogenic) effects of pesticides on human health have been difficult to detect. Perhaps the health risks are sufficiently small that they are below the power of epidemiologic studies to detect. But it is also possible that there are very few effects to humans at all. While a comprehensive review of the epidemiologic studies conducted world-wide on pesticides, fertilizers, and/or agricultural practices (e.g., poultry, hog, dairy production) yielded conflicting results, the limitations of epidemiologic studies were also given (1) as shown in the following quotation.

> "Epidemiologic analysis of data in man often fails to yield conclusive results because the size of the study group is too small (statistical power is inadequate to detect a difference), exposure data are lacking, concomitant or prior exposures to other known or suspected carcinogens may interact with the compound(s) in question and confound the analysis, or a proper control group cannot be identified. Then, too, some well-defined studies in different groups under different circumstances may yield totally conflicting findings."

Even if there were no such limitations, epidemiologic analysis is retrospective study; it cannot be depended upon for the detection and prevention of potential health hazards to the public. In comparison to epidemiologic studies, animal bioassays are short in duration, relatively inexpensive, easily perfomed under controlled conditions, and are reliable predictors for known human carcinogens. Therefore, from the point of view of identifying and preventing public health hazards, animal bioassay is an invaluable tool.

As of April 1989, the National Toxicology Program (NTP) and the National Cancer Institute (NCI; up to 1981), have completed chronic toxicity/carcinogenicity studies on 385 chemicals (2). Of the chemicals studied, 63 were considered pesticides (General or Unclassified) (Tables I and II). Approximately 41% (26/63) of the pesticides eva-

Table I. NCI/NTP Carcinogenicity Study Results for Pesticides: Carcinogenic Pesticides Under the Experimental Conditions

Chemcial Name	TR No.	Route	Carcinogenicity Results			
			MR	FR	MM	FM
Aldrin	021	Feed	E	E	P	N
Captan	015	Feed	N	N	P	P
Chloramben	025	Feed	N	N	E	P
Chlordane	008	Feed	N	N	P	P
Chlorobenzilate	075	Feed	E	E	P	P
3-Chloro-2-methylpropene	300	Gav	CE	CE	CE	CE
Chlorothalonil	041	Feed	P	P	N	N
Daminozide	083	Feed	N	P	E	N
1,2-Dibromo-3-chloropropane (DBCP)	028	Gav	P	P	P	P
	206	Inh	P	P	P	P
1,2-Dibromoethane (EDB)	086	Gav	P	P	P	P
	210	Inh	P	P	P	P
1,4-Dichlorobenzene	319	Gav	CE	NE	CE	CE
1,2-Dichloropropane	263	Gav	NE	EE	SE	SE
1,3-Dichloropropene (Telone II)	269	Gav	CE	SE	IS	CE
Dichlorvos	342	Gav	SE	EE	SE	CE
Dicofol	090	Feed	N	N	P	N
Ethylene oxide	326	Inh			CE	CE
Heptachlor	009	Feed	N	E	P	P
Mirex	313	Feed	CE	CE		
Monuron	266	Feed	CE	NE	NE	NE
Nitrofen	184	Feed	N	N	P	P
	026	Feed	IS	P	P	P
Piperonyl sulfoxide	124	Feed	N	N	P	N
Sulfallate	115	Feed	P	P	P	P
Tetrachlorovinphos	033	Feed	N	P	P	P
Toxaphene	037	Feed	E	E	P	P
2,4,6-Trichlorophenol	155	Feed	P	N	P	P
Trifluralin	034	Feed	N	N	N	P

TR = NTP Technical Report; Gav = Gavage; Inh = Inhalation; MR = Male rats; FR = Female rats; MM = Male mice; FM = Female mice

For experiments evaluated by the NCI or the NTP prior to June 1983, results are reported as "positive" (P), "negative" (N), "equivocal" (E), or "inadequate" (IS). In June 1983, the NTP adopted the use of "categories of evidence": two of the five categories correspond to positive results ["clear evidence" (CE) and "some evidence" (SE) of carcinogenicity], one is for uncertain findings ["equivocal evidence" (EE)], one is for negative studies ["no evidence" (NE)], and one is for studies that cannot be evaluated because of major flaws ["inadequate studies" (IS)].

Table II. NCI/NTP Carcinogenicity Study Results for Pesticides: Non-carcinogenic Pesticides Under the Experimental Conditions

Chemcial Name	TR No.	Route	Carcinogenicity Results MR	FR	MM	FM
Aldicarb	136	Feed	N	N	N	N
Anilazine	104	Feed	N	N	N	N
Azinphosmethyl	069	Feed	E	N	N	N
Calcium cyanamide	163	Feed	N	N	N	N
2-Chloroethyltrimethylammonium chloride	158	Feed	N	N	N	N
Chloropicrin	065	Gav	IS	IS	N	N
Clonitralid	091	Feed	N	E	IS	N
Coumaphos	096	Feed	N	N	N	N
Diazinon	137	Feed	N	N	N	N
Dichlorvos	010	Feed	N	N	N	N
Dieldrin	021	Feed	N	N	E	N
	022	Feed	N	N		
1,2-Dichlorobenzene	255	Gav	N	N	N	N
Dichlorodiphenyldichloroethane (TDE)	131	Feed	E	N	N	N
Dichlorodiphenyltrichloroethane (DDT)	131	Feed	N	N	N	N
Di(p-ethylphenyl) dichloroethane (DDD)	156	Feed	N	N	N	E
Dimethoate	004	Feed	N	N	N	N
Dioxathion	125	Feed	N	N	N	N
Endosulfan	062	Feed	IS	N	IS	N
Endrin	012	Feed	N	N	N	N
Fenthion	103	Feed	N	N	E	N
Fluometuron	195	Feed	N	N	E	N
Lindane	014	Feed	N	N	N	N
Malaoxon	135	Feed	N	N	N	N
Malathion	024	Feed	N	N	N	N
	192	Feed	N	N		
Methoxychlor	035	Feed	N	N	N	N
Methyl parathion	157	Feed	N	N	N	N
Mexacarbate	147	Feed	N	N	N	N
Parathion	070	Feed	E	E	N	N
Pentachloronitrobenzene	061	Feed	N	N	N	N
	325	Feed			NE	NE
o-Phenylphenol	301	SP			NE	NE
Phosphamidon	016	Feed	E	E	N	N
Photodieldrin	017	Feed	N	N	N	N
Picloram	023	Feed	N	E	N	N
Piperonyl butoxide	120	Feed	N	N	N	N
Rotenone	320	Feed	EE	NE	NE	NE
2,3,5,6-Tetrachloro-4-nitroanisole	114	Feed	N	N	N	N
Triphenyltin hydroxide	139	Feed	N	N	N	N

SP = Skin paint; For all other abbreviations, see Table I footnotes

luated showed varying degrees of carcinogenicity in animal studies (2); these carcinogenic pesticides and those found to be non-carcinogenic in the test systems are presented in Tables I and II, respectively. The criteria used to determine whether a pesticide is carcinogenic are according to the NTP or NCI convention; that is: one or more of the four experiments (i.e., male rats, female rats, male mice, female mice) showed "clear evidence", "some evidence" of carcinogenicity, or was ruled to be "positive" for carcinogenicity (3,4). A caution must be made here that the percentage (i.e., 41%) for pesticides tested positive as carcinogens does not mean that the same percentage will hold true for all pesticides or all chemicals. The fact that these chemcials were nominated to the NCI or NTP for testing means there was concern that these chemicals could be potentially troublesome; thus the sampling is slanted toward potential carcinogens.

As shown in Figure 1, the primary efforts of the NTP center on chemical nomination, evaluation of the existing information, study design and conduct, data analysis and interpretation, and finally the preparation, public peer-review, and publication of technical reports. However, the information presented in the NTP Technical Report is frequently used world-wide for hazard identification and risk assessment, often leading toward regulatory decisions and actions. Even though the NTP is not directly involved in the regulatory processes, the data generated at the NTP play an important role in these processes.

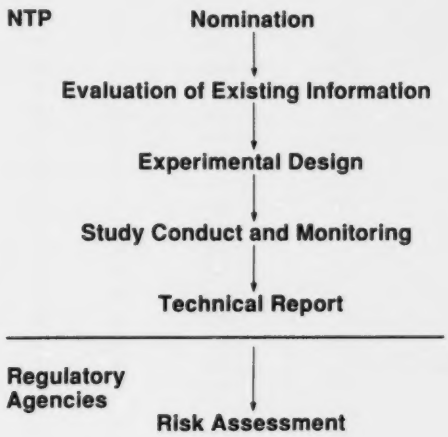

Figure 1. Flow chart of the functions of the NTP in the toxicologic characterization of chemicals and its role in relation to regulatory agencies.

Once a chemical is demonstrated to have carcinogenic activity in laboratory rodents, extrapolations from "animal carcinogen" to "human carcinogen" and from "high dose" (i.e., the dose used in animal studies) to "low dose" (i.e., the dose humans are exposed to) are inevitable in the risk assessment process. Many biological issues are automatically brought into the limelight. At least some of these issues had been "locked in" as early as the experimental design stage. Those issues most often discussed are species, strain, sex, dose, route, and to some extent exposure scenario. Though important in the risk assessment process, the issues of toxicological interactions and the health effects of chemical mixtures have received comparatively little attention. In this paper, we will only discuss the current thoughts on a few selected issues with a special emphasis on toxicological interactions and chemical mixtures.

Species Extrapolation

Are rats and mice good models for humans toxicologically? This is an age-old question and opinions continue to be varied. But "Is a human a good model for humans?" Assuming there are no ethical and moral considerations and we, as a society, could perform toxicological studies including carcinogenicity bioassays in humans, would there be arguments? The answer is most likely a yes!, because there are so many environmental and life style differences (e.g., dietary preference, smoking, coffee drinking, alcohol indulgence, exercise, etc.) among individuals that the outcome of the results may easily be influenced. Assuming further that we built "human cages" with exercising machines, formulated "NIH-07 Human Chow," and completely eliminated all environmental and life style variables, would we then be more comfortable? The answer is no because there are known human pharmacogenetic differences such as fast acetylators and slow acetylators, and other biochemical and physiological diversities. There will always be arguments such as "Why didn't you use a different subpopulation?"

The point is that there is not and will never be a perfect system and that we have to settle for less than perfect. The right question to ask then is "Given the fact that there is no perfect system, how good are rats and mice as models for humans in toxicology?" Reviewing the literature regarding the development of the carcinogenicity bioassay, one finds such discussion as the one given by Shimkin (5) who proposed a general set of criteria for the selection of test animals in carcinogenicity studies: (a) availability; (b) economy; (c) sensitivity to carcinogens; (d) stable as to response; (e) similarity to human in regard to metabolism; (f) similarity to human in regard to pathology responses. When all these criteria are considered realistically, not too many laboratory animal species may be used for carcinogenicity studies. Other examples in the literature on the selection of species for carcinogenicity bioassay include Sontag (6) and Weisburger and Weisburger (7). On the basis of certain criteria, Sontag (6) suggested that the only

species suitable for large scale, long term carcinogenicity bioassays are the rat, mouse, and hamster. Weisburger and Weisburger (7), on the other hand, indicated that when specific questions are asked of the bioassay rather than the simple endpoint of the increases of neoplasms, a variety of species can be used. Thus, the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation suggested examples where the specific relationship of a highly defined genetic background and the induction of neoplasms in animals harboring few oncogenic viruses may lead to the use of fish or insects (8). In our opinion, considering the scientific as well as practical issues, laboratory rodents are still the best models available to provide information regarding the potential toxicological consequences of chemicals in question. As responsible scientists in the public health arena, we do not have the luxury to ignore the results from animal studies even if they are less than perfect models for humans. For instance, in recent years, there has been higher prevalence of fin rot, tumors and other lesions in Winter Flounders caught in the Boston Harbor (9). Are we to take such findings as a valid warning from nature and consider them as the results of a "natural bioassay" on the pollution in the area, or simply ignore it on the basis that a fish is not a human?

There is abundant debate in the literature about the use of laboratory rodents as models for humans; a recent example appeared in Statistical Science (10-16). Some scientists considered rodents very good surrogates for humans in predicting carcinogenicity; for instance, of the known human carcinogens, 86% to 100% (depending on the data base used) were shown to be animal carcinogens (12,17-19). Others (10), apparently using the same data base but only the "sufficient evidence" category as defined by the International Agency for Research on Cancer (IARC) (18) in their calculation, considered rodents to be poor models for humans because the concordance was poor (59%). At issue though is not whether or not the concordance is 100% or 59% between human and animal models because even a selected sample of humans probably do not have 100% concordance with the rest of the human population. We know laboratory rodents are not perfect surrogates for humans but they definitely provide valuable scientific information regarding the intrinsic biological activities of chemicals.

It is important for scientists in toxicology to recognize the imperfection of the animal bioassays and to try to continually develop better scientific methods to minimize the differences in the extrapolation between species and between high and low doses. As a case in point, the incorporation into the risk assessment process of physiologically based pharmacokinetics/computer modeling which takes into consideration physiological constants (e.g., body size, organ and tissue volumes, blood flow, and ventilation rates), biochemical constants (e.g., metabolic constants, partition coefficients for blood, tissues and air), and mechanistic factors (e.g., target tissues and metabolic pathways) of various species, including human, represents a major scientific advance in recent years (20).

The importance of continuing refinement and improvement of the hazard identification and risk assessment process based on newly developed scientific information may be illustrated by another example using a pesticide, EDB or ethylene dibromide. Critics of cancer risk assessment based on animal bioassays frequently make reference to a paper by Ramsey et al. (21). In that paper (21), Ramsey and coworkers questioned the validity of the EPA's estimate of 100% lifetime incidence of cancer to be expected in humans exposed to a concentration of 0.4 ppm (3.1 mg/m^3) of EDB for 40 years. As pointed out by Ramsey et al. (21), the reliability of such a prediction is dependent upon the validity of the carcinogenesis model (one-hit model in this case) as well as its extrapolation from rat bioassay data to human population. To test these hypotheses, Ramsey et al. (21) compared the incidence of cancer predicted by the EPA one-hit carcinogenic model with that observed in a group of 156 workers employed in the production of EDB. While the one-hit model predicted a total of 85 tumors above the normal background incidence (2.2 tumors expected based on a comparison with the U. S. white male mortality rates) in this group of workers, the actual tumor incidence rate of 8 tumors was observed (21). Ramsey et al. concluded that use of the one-hit model appears to result in "highly exaggerated" risk estimates. Their results and conclusions have been cited as evidence that extrapolations from animal bioassays to human real-world exposures are implausible. A more recent attempt by Hertz-Picciotto et al. (22), however, has provided counter arguments. When cancer risks for EDB among the cohort of workers used by Ramsey et al. (21) were estimated by fitting several linear nonthreshold additive models to data from gavage and inhalation animal bioassays (22), the predicted upper bound risks were within a factor of 3 of the observed cancer deaths (Table III). Thus, Hertz-Picciotto et al. (22) concluded that the previous overestimate of risk to workers occupationally exposed to EDB was due to failure to consider their age at start of exposure when extrapolating from an animal bioassay with an exceedingly short latency period. In the quotation from Hertz-Picciotto et al. (22) below, the spirit of continuing improvement and refinement of the hazard identification and risk assessment process is elegantly reflected:

> "The field of carcinogenic risk assessment is in its infancy. The primitiveness of methodology echoes the lack of a clear theory of carcinogenesis. However, the gaps in knowledge and the uncertainties in methods do not constitute sufficient justification for abandoning efforts to provide the public with plausible upper bounds for cancer risks due to environmental chemical exposures. For a large number of such exposures, these estimates will necessarily be based on animal data. When quantified human exposure data are available and are related to cancer risk, these data can be useful either as a basis for extrapolation or as a standard for assessing the plausibility of risk estimates based on animal data alone."

Table III. Total Cancer Deaths Predicted by Several Models for EDB-Exposed Workers

	Observed cancer deaths	Models fitted to gavage data					Model fitted to Inhalation data	
			Proportional hazards				One-hit: terminal sacrifice tumors	
		One-hit	Stomach	Lung	Multistage with time-to-tumor	Multistage with variable dosing	Omitted	Included
					0.9 ppm Exposure			
Texas	3	38.6	10.4	31.4	7.7	7.1	3.8	4.0
Michigan	5	21.2	5.9	13.0	3.5	4.5	2.3	2.4
Overall	8	59.8	16.3	44.4	11.2	11.6	6.1	6.4
					3 ppm Exposure			
Texas	3	56.6	21.4	50.4	15.5	14.0	4.2	4.9
Michigan	5	34.2	11.7	24.3	6.1	7.7	2.5	2.9
Overall	8	90.8	34.5	74.7	21.6	21.7	6.7	7.8

Condensed from Hertz-Picciotto (22)

Maximum Tolerated Dose (MTD)

Since quantitative risk extrapolation is presented in another chapter, we discuss briefly here the issue of maximum tolerated dose (MTD). Actually "maximum tolerated dose" is a rather nebulous term without specific qualifiers. A generally accepted definition does not exist. The definition for MTD, given in one source (8), is "...this dose is determined by prechronic studies which aid in the identification of a dose level which, when given for the duration of the chronic study as the highest dose, will not impair the normal longevity of the treated animals from effects other than the induction of neoplasms. Such dose should not cause morphologic evidence of toxicity in organs other than mild changes such as slight hypertrophy or hyperplasia, inflammation or slight changes in serum enzymes..." In a second source (23), MTD is attributed to be the result of "...the simplistic approach of the National Cancer Institute's Bioassay Program, which is to conduct a 3 month range finding study with enough doses to find a level which suppresses body weight gain slightly, i.e., 10%. This dose is defined as the maximum tolerated dose (MTD) and is selected as the highest dose." In a third source (24), MTD is defined as "...a predictive dose obtained from analysis of subchronic study doses. In the oncogenicity study, the MTD should elicit toxicity without substantially altering the normal life span of the test species from effects other than tumor formation." In this case, it is of course really an exercise after the fact (i.e., after the completion of the chronic toxicity study) to evaluate if the doses selected were reasonable. Examples in such an evaluation for determining a MTD include body and organ weight effects as well as clinical and anatomic pathology (24). Thus, MTD must be considered in the context of the toxicological endpoints measured.

In the estimation of MTD and setting doses for chronic toxicity/carcinogenicity studies, one often hears advice against selecting any dose within the range of "saturation kinetics" (or "nonlinear kinetics"). The underlying reason for such advice is that we, the experimenters, should not "overwhelm" the animals' capacity of handling chemical insults. This is once again debate or argument resulting from nebulous terms. If one considers that any toxicity or toxicological response(s) is a manifestation of the disruption of homeostasis, then somewhere in the body there must be a system(s) (e.g., sites of action, transport mechanisms, metabolic pathways, binding sites, repair mechanisms, etc.) being overwhelmed. In fact, it has been suggested that toxicology is nonlinear pharmacokinetics (25). Therefore, at the stage of selecting doses for chronic toxicity studies based on prechronic toxicity data, it is very difficult to talk about nonlinear pharmacokinetics or saturation kinetics without defining precisely what parameters are involved.

As to the debate on the use of estimated MTD in chronic toxicity/carcinogenicity studies, recent information may be found in two letters by Abelson (26) and McConnell (27). Abelson suggests that the public has been misinformed through "a media barrage" by the results of chemical carcinogenesis studies in animals, particularly in rodents (26). One of the criticisms from Abelson was the use of "massive doses" (i.e., MTD) which, in his opinion, "vastly exceed those to which humans are likely to be exposed" (26). McConnell

(27), on the other hand, pointed out that, in at least three important instances (asbestos, benzene, and 1,3-butadiene), past occupational exposures are not different from the levels used in laboratory experiments. Further, McConnell (27) defended the use of MTD in carcinogenicity studies because animals are able to detoxify chemicals much faster than humans do, and to get an equivalent dose to the target tissue in such a case would require much more of the chemical in animals. He illustrated his point by using cigarette smoke as an example; it takes an unusually high exposure of cigarette smoke to cause cancer in laboratory aniumals as compared to humans (27). McConnell asked that if cigarette smoke were an "unknown," would one assume tobacco was safe because the dose to animals was higher than the average human receives? (27).

Toxicologic Interactions

Toxicologic interaction has been defined as "a circumstance in which exposure to two or more chemicals results in a qualitatively or quantitatively altered biological response relative to that predicted from the actions of the single chemicals independently. The multiple-chemical exposures may be simultaneous or sequential in time, and the altered responses may be greater or smaller in magnitude" (28). While most if not all of the known examples of toxicologic interactions concerning pesticides (29) involve respective chemicals at concentrations far above levels of environmental contamination, recent findings have uncovered toxicologic interactions at low enough concentrations to be relevant to environmental pollutions. Table IV illustrates an example of toxicologic interaction other than the classical instances of insecticide synergism (e.g., pyrethrin and piperonyl butoxide). Klingensmith and Mehendale (30) and Mehendale (31) reported probably the first case where a pesticide, Kepone, at an environmentally realistic level (i.e., 10 ppm) caused a dramatic increase (67-fold) in the acute toxicity of carbon tetrachloride. Although this synergistic effect of Kepone is rather specific in that close structural analogs such as mirex and photomirex do not share this property (31), one wonders whether other chemical(s) or chemical mixtures, at environmentally low levels, might cause similar synergistic toxicity. Indeed, the NTP has obtained some preliminary experimental results (presented later) which may shed light upon this question.

Toxicologic interactions may occur in chronic toxicity and carcinogenicity studies. For instance, Wong and colleagues (32) demonstrated a profound enhancement of mortality, tumor incidences and the shortening of latency period for neoplasms of a well-known fumigant, EDB, by disulfiram (antabuse) in a chronic toxicity study. As shown in Table V, the numbers and the types of tumors increased significantly when EDB and disulfiram were given together. Of course, the reason for such a synergistic effect was due to the interference of metabolic degradation of EDB by disulfiram (32). The relevance of this study, as was the original purpose for the investigation (32), is the concern of the potential synergistic hazard for people occupationally exposed to EDB (e.g., production workers, pest control applicators) who might be simultaneously participating in an alcohol control program using antabuse as a therapeutic agent.

Table IV. Enhancement of Acute Toxicity of Carbon Tetrachloride by Low Level Dietary Pretreatment of Kepone

Dietary Pretreatment	48-hr LD_{50} (ml CCl_4/kg)	Increase in Mortality
Control diet	2.8	--
Kepone (10 ppm) diet	0.042	67-fold

Condensed from Klingensmith and Mehendale (30) and Mehendale (31)

These examples above demonstrate yet another area of uncertainty in the extrapolation of animal toxicity studies to the hazard identification and risk assessment of humans. Since the data base in this area is extremely limited, particularly at chemical concentrations which are environmentally realistic, major endeavours in this area are urgently needed.

Table V. Major Histopathological Findings in Rats Exposed to EDB or EDB/Disulfiram (EDB+DS) in the EDB/Disulfiram Interaction Study

	EDB		EDB+DS	
	Male	Female	Male	Female
No. of Animals Examined	46	48	48	45
Liver hepatocellular tumors	2	3	36*	32*
Mesentary or omentum hemangiosarcoma	0	0	11*	8*
Kidney adenoma and adenocarcinoma	3	1	17*	7*
Thyroid follicular epithelial adenoma	3	1	18*	18*
Mammary all tumors	---	25	---	13*
Lung all tumors	3	0	9*	2
No. of Rats with Tumor	25	29	45*	45*
No. of Rats with Multiple Tumors	10	8	37*	32*

* $P<0.05$

Condensed from Wong et al. (32). Disulfiram was given in diet at 0.05% and EDB inhalation exposure was at 20 ppm. The control and DS alone groups (not shown here) had, in general, very low or no tumor incidence with respect to these organs; the only exception was that the DS alone group had statistically higher incidence in mammary tumors than did the control group.

Toxicology of Chemical Mixtures

Human exposure to chemicals, be it occupational or environmental, is rarely limited to a single chemical. Even in a strictly controlled situation such as a production plant for a single chemical, the exposure of workers to a variety of other chemicals in food, drink, personal hygiene, etc. is part of daily life. As illustrated very clearly in a recent government publication on human health and the environment (33), "...Each of us is exposed daily to multiple chemical substances in our environments. The food we eat is a complex mixture of chemical substances. Our drinking water contains hundreds of chemicals, even when it is obtained from a municipal "purification" facility. The air we breathe and the things we touch likewise contain a variety of chemicals with an almost limitless range of compositions. Moreover, our habits and lifestyle may add other chemical exposures. Tobacco smoke, for example, contains thousands of substances. Additionally, the American public spends between $4 billion and $8 billion per year on self prescribed and self-administered drugs for self-diagnosed illness..."

In the last few years, the NTP has been interested in the toxicology of a mixture of 25 frequently occurring groundwater contaminants derived from hazardous waste disposal sites. The diagram in Figure 2 is a summary of the projects completed or ongoing at the NTP and neighbouring institutes. The genesis of such a program, the formulation of a 25-chemical (19 organics and 6 inorganics) mixture in deionized water, and the analytical and initial animal toxicology work have been reported (34-41). In most of our animal studies, the highest dose level, as shown in Table VI, contains individual chemical concentrations close to those detected in the groundwater samples near hazardous waste disposal sites. When this mixture is given to female B6C3F$_1$ mice for 14 or 90 days, suppression of immune function was seen in 3 of the parameters examined (38); the results are summarized in Table VII. First, the suppression of bone marrow stem cell proliferation, as expressed by the number of colonies formed of the granulocyte-macrophage progenitor cells is presented. Note the lack of response in the paired-water control group in the 14-day study; this finding suggests that the immunotoxic responses seen had little, if anything, to do with the reduction of water consumption in the treated groups. A clear dose-response relationship was demonstrated in the 90-day study results on the suppression of bone marrow stem cell proliferation. The second immunological endpoint affected is the suppression of antigen (sheep red blood cell)-induced antibody forming cells (Table VII); similar results as discussed above for the stem cell suppression are also evident for this endpoint. Three host resistance assays following challenge with infectious agents (Listeria monocytogens, PYB6 syngeneic tumor cells, or Plasmodium yoelii) were investigated (38). Altered resistance, as expressed by % parasitemia, occurred in the group challenged with Plasmodium (Table VII). These results collectively suggest that long-term exposure to heavily contaminated groundwater may represent a risk to the immune system (38).

In another experiment (Figure 3), a collaborative effort between the EPA and the NTP, we examined the effects of pretreatment with the 25-chemical mixture of groundwater contaminants for 14 days on the hepatotoxicity of carbon tetrachloride in male Fischer 344 rats (39).

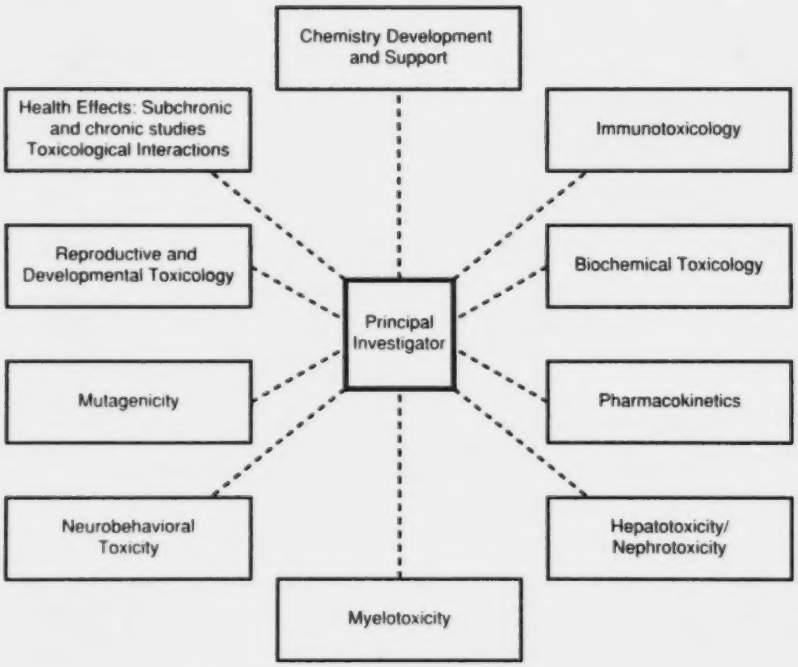

Figure 2. A program of toxicology of complex chemical mixtures of groundwater contaminants at the NTP.

Table VI. Comparison of Concentrations of the Components of the 25 Chemical Mixture Dosing Solution with EPA Survey Results

	A	B	
	Ave EPA Survey ppm	High Dose Concentrations (ppm)	Ratio B/A
Acetone	6.9	53	7.7
Arochlor 1260	0.21	0.01	0.05
Arsenic	30.6	9	0.29
Benzene	5.0	12.5	2.5
Cadmium	0.85	51	60
Carbon tetrachloride	0.54	0.4	0.74
Chlorobenzene	0.1	0.1	1
Chloroform	1.46	7	4.79
Chromium	0.69	36	52.2
DEHP	0.13	0.015	0.12
1,1-Dichloroethane	0.31	1.4	4.52
1,2-Dichloroethane	6.33	40	6.32
1,1-Dichloroethylene	0.24	0.5	2.08
1,2-trans-Dichloroethylene	0.73	2.5	3.42
Ethylbenzene	0.65	0.3	0.46
Lead	37.0	70	1.89
Mercury	0.34	0.5	1.47
Methylene chloride	11.2	37.5	3.35
Nickel	0.5	6.8	13.6
Phenol	34.0	29	0.85
Tetrachloroethylene	9.68	3.4	0.35
Toluene	5.18	7	1.35
1,1,1-Trichloroethane	1.25	2	1.6
Trichloroethylene	3.82	6.5	1.7
Xylenes	4.07	1.6	0.39

3 chemicals	> 10X	Average EPA Survey Concentration
14	1X - 10X	
8	< 1X	

As Table VIII demonstrates, the deionized water control group and the drinking water chemical mixture alone group showed no histopathological changes in the liver. While the CCl_4 alone group showed a mild centrilobular vacuolar degeneration at the dose level applied (0.075 ml/kg), the mixture/CCl_4 group showed, in addition to the mild centrilobular vacuolar degeneration, minimal centrilobular hepatocellular necrosis (Table VIII). The necrotic changes in the liver in the mixture/CCl_4 group also coincided with the elevation of serum aspartate aminotransferase (177% of controls) and alanine aminotransferase (273% of controls). We are currently conducting an experiment to determine the effect of prior exposure to the 25-chemical mixture on the CCl_4 dose-response curve and to determine the influence of the observed decrease in water and feed consumption on the apparent enhancement of CCl_4 hepatotoxicity.

Table VII. Immune Functions in B6C3F$_1$ Mice Affected by Exposure to a Chemical Mixture of Groundwater contaminants

Exposure Level (% Stock)	CFU-GM /10^5 cells1	PFC/Spleen (X 10^3)2	P. yoelii % parasitemia3
		14-day study	
0	59.8 ± 6.0	172 ± 16	14.2 ± 1.2
0.2	57.2 ± 1.3	231 ± 16	9.2 ± 1.0
2.0	55.4 ± 3.2	157 ± 24	19.8 ± 2.9
20.0	37.8 ± 0.4**	96 ± 16**	22.1 ± 2.9*
Paired water	50.5 ± 1.3	172 ± 17	N.D.
		3-month study	
0	55.3 ± 2.1	189 ± 49	10.1 ± 1.5
1.0	52.6 ± 2.9	120 ± 19	9.4 ± 2.9
5.0	43.5 ± 4.6*	144 ± 21	12.7 ± 1.9
10.0	29.6 ± 1.7**	93 ± 15	20.8 ± 2.9*

1 The CFU-GM, granulocyte-macrophage colonies, were assayed by incubating femoral bone marrow cells in the presence of mouse lung conditioned medium as a colony stimulating factor at 37°C in 5% CO_2 for 7 days. Colonies of >50 cells were enumerated using a stereomicroscope. Values given represent mean ± SE of CFU-GM per 10^5 cells for at least five mice per group.

2 The antibody response to sheep erythrocytes was determined by enumerating plaque-forming cells (PFC) in splenic lymphocytes 4 days after primary immunization. Values given represent mean ± SE of PFC per spleen for at least five mice per group.

3 Infection with the malarial parasite *P. yeolli* was determined by quantitating the percent parasitemia on days 10, 12, and 14 following injection of 10^6 parasitized erythrocytes. Only peak day, day 12, of infection is shown. Values given represent mean ± SE of eight mice per group.

N.D. = not done;
** Significantly different from control at $P < 0.01$;
* Significantly different from control at $P < 0.05$

SOURCE: Data are from ref. 38.

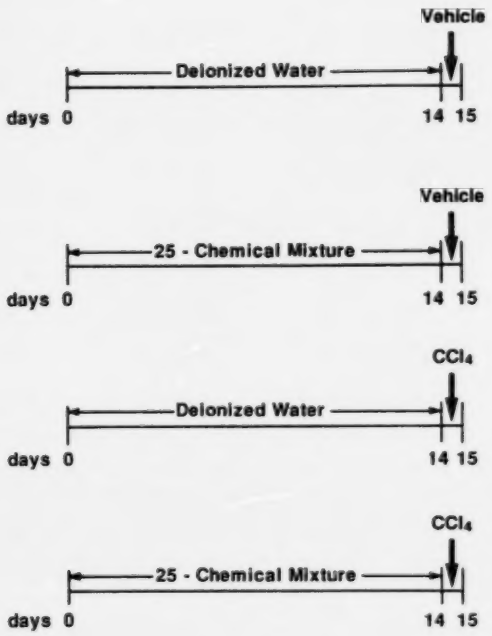

Figure 3. Experimental design of the toxicologic interaction study between a chemical mixture of 25 groundwater contaminants and carbon tetrachloride.

Table VIII. Enhancement of Carbon Tetrachloride Hepatotoxicity in Male Fischer 344 Rats by Prior Exposure to a Mixture of 25 Groundwater Contaminants

Treatment[1] Groups	No. Animal Examined	Clinical Chemistry Parameters		Liver Histopathology (No. Rats with Lesion)		
		AST2	ALT2	Normal	Vacuolar Degeneration	Cellular Necrosis
Control/ Control	4	52.8 ± 4.3	35.0 ± 4.1	4	0	0
Low Mixture/ Control	4	46.5 ± 2.6	32.8 ± 1.7	4	0	0
High Mixture/ Control	4	53.8 ± 6.2	39.0 ± 2.2	4	0	0
Control/ CCl_4	4	55.0 ± 3.9	47.0 ± 7.5	0	4	0
Low Mixture/ CCl_4	4	52.5 ± 4.0	41.2 ± 7.4	0	4	0
High Mixture/ CCl_4	4	93.5 ± 36.5**	95.5 ± 49.7**	0	4	3

1 The rats were given deionized water (Control) or 1% (Low Mixture) or 10% (High Mixture) mixture stock for 14 days and then dosed by gavage a single dose of corn oil (Control) or CCl_4 at the rate of 0.075 ml/kg in corn oil; the animals were then sacrificed 24 hours later.

2 AST = Aspartate aminotransferase; ALT = Alanine aminotransferase

**Significantly different from the Control/Control group, the High Mixture/Control group, and the Control/CCl_4 group, $p < 0.01$.

The primary hazard of pesticide exposure is acute toxicity resulting from dermal contact with or inhalation of a relatively large dose (1). However, the toxicology of chemical mixtures at the level of environmental contamination will probably not involve acute toxic responses. It is most likely an insidious effect(s) disrupting the homeostasis of the organism. The exposed animals may appear totally "normal" clinically or based on conventional toxicological endpoints. However, such a subclinical state may provide a basis for enhancement or potentiation of otherwise mild toxic responses from an acute exposure(s) of chemical, physical, and/or biological agents. In this sense, the concept of a generic "promotor" or "enhancer" for any possible toxicity may be advanced for the potential toxicologic consequence of a mixture of environmental pollutants. These findings also raise the possibility for synergistic interaction between a background long-term, low-level chemical mixture exposure and an subsequent acute dose resulting from accidental exposure or drug intake including alcohol abuse. All these issues are not only relevant but very important in the extrapolation between animals and humans.

Concluding Remarks

Two years ago, in the ACS symposium on "Pesticides: Minimizing the Risks", the utilization of physiologically based pharmacokinetics/computer modeling for dealing with the complex issues of extrapolation, toxicological interaction, and chemical mixtures was suggested (42,43). As shown in Figure 4, the Stage I effort has already been

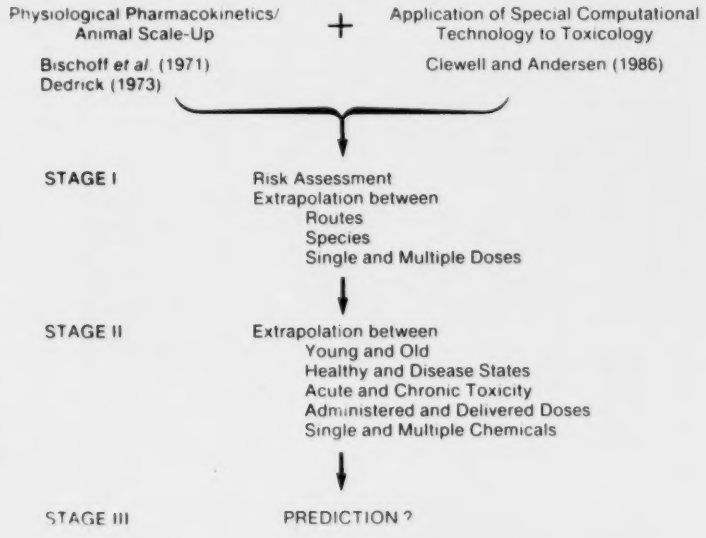

Figure 4. A suggested approach utilizing physiologically based pharmacokinetics and computer technology for the extrapolation and prediction of various situations in toxicology. (Reproduced from ref. 42. Copyright 1987 American Chemical Society.)

in progress. Some scientific activities are also evident for the effort of Stage II and beyond (44-49). In the last two years, a great deal of progress has been made in this area including a Workshop on "Pharmacokinetics in Risk Assessment" organized by the National Research Council which resulted in the publication of volume 8 of the Drinking Water and Health series (20). However, like any other new technique, the computer modeling of physiologically based pharmacokinetics and its application to risk assessment is going through an evolutionary phase. Much debate and controversy have been appearing in the literature. As a case in point, EPA's adoption of physiologically based pharmacokinetics in their risk assessment process and the related criticisms and debate reflect the current state of flux (48,49). Nevertheless, all these activities and debates will inevitably help to improve and refine this approach. Eventually, the scientific community and the public at large will have a better risk assessment method.

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